

--Example 1. Preparation of *P. insidiosum* lipid

In an 80 liter (gross volume) fermentor, 51 liters of tap water, 1.2 kg glucose, 240 grams of yeast extract and 15 ml of MAZU 210S® antifoam were combined. The fermentor was sterilized at 121°C for 45 minutes. An additional 5 liters of condensate water were added during the sterilization process. The pH was adjusted to 6.2, and approximately 1 liter of inoculum (at a cell density of 5-10g/l) of Pythium insidiosum (ATCC #28251) then was added. The agitation rate was adjusted to 125 RPM (250 cm/sec tip speed) and the aeration rate was set at 1 SCFM (standard cubic feet per minute). At hour 24 in the operation the aeration rate was increased to 3 SCFM. At hour 28 an additional 2 liters of 50% glucose syrup (1 kg glucose) were added. At hour 50 the fermentor was harvested, resulting in a yield of about 2.2 kg wet weight (approximately 15 g dry weight) per liter. Harvested biomass was squeezed to a high solids cake (50% solids) on a suction filter before freeze drying. The dried biomass was ground with a mortar and pestle and extracted with 1 liter of hexane per 200 grams of dry biomass at room temperature under continuous stirring for 2 hours. The mixture then was filtered and the filtrate evaporated to yield about 5-6 grams of crude oil per 100 grams of dry biomass. The biomass then was reextracted with 1 liter of ethanol per 20 grams of dry biomass for 1 hour at room temperature, filtered, and the solvent evaporated yielding an additional 22 grams of crude oil per 100 grams of dry biomass. The second fraction was predominantly

phospholipids whereas the first fraction contained a mixture of phospholipids and triglycerides. The combined fractions produced an oil containing about 30-35% arachidonic acid and no detectable EPA.

Example 2. Preparation of *M. alpina* lipid

Mortierella alpina (ATCC #42430) was grown in a 2 liter shake flask containing 1 liter of tap water and 20 grams of potato dextrose medium. The flask was under constant orbital agitation and was maintained at 25°C for seven days. After harvesting by centrifugation, the biomass was freeze dried yielding about 8 grams of lipid-rich mycelia. The mycelia was extracted using hexane as in example #1 and about 2.4g of crude oil resulted. This oil contains about 23% arachidonic acid.

Example 3

Into a 30-liter working volume STF was loaded a medium of one quarter strength artificial seawater. Six liters of IO were combined with 18 liters of tap water. The fermentor containing the medium was sterilized and cooled to 28°C. Four hundred ml of concentrated YE (455g/l), 900 ml of glucose syrup (400 g/l) and one liter of inoculum from a seed fermentor containing about 2×10^7 *C. cohnii* cells/ml or a biomass of 20g/liter (yielding a final concentration of about 10^5 cells/ml of a biomass of about 700 mg/liter), were added to the medium. The *C. cohnii* cells, designated MK8840, were obtained from the American Type Culture Collection as ATCC 40750. Agitation was

set at 120 cm/sec tip speed and aeration was set at 1 VVM (30 liters per minute). Additional glucose syrup (900 ml) was added after 30 hours and another 4.2 liters over the next 42 hours. Thus 6 liters of glucose syrup were added in total. Concentrated YE solution (400 ml) was added at hour 6 another 1.2 liters were added over the next 48 hours until a total of 2.0 liters had been added. To maintain the D.O. at greater than 20%, at 24 hours the agitation tip speed was increased to 150 cm/sec and at 48 hours to 160 cm/sec. At 72 hours, the tip speed was increased to 200 cm/sec and the culture was permitted to grow for an additional time sufficient to convert the final charge of glucose into cellular oil. The culture was then harvested by centrifugation with the cell pellet retained. The harvested pellet of cells was frozen and dried (lyophilized) to about a 4% moisture content. Hexane (2.8 liters) was added to the dried biomass and stirred in a glass kettle for 1.5 hours at 50°C. A rotary evaporate was used to remove the hexane, producing about 175 g of crude DHA-containing oil.--

At page 11, line 28 after "Example", change "1" to --4--.

At page 12, lines 8-9 kindly delete "patent application # 07/479-135" and substitute therefore --Example 3--.

At page 12, line 13 kindly delete "patent application # _____" and substitute therefore --Example 2--.

At page 14, line 1 after "Example", change "2" to --5--.

At page 14, line 8 delete "patent application 07/479-135" and substitute therefore --Example 3--.

At page 14, line 11 delete "patent application ____" and substitute therefore --Example 2--.

At page 14, line 14, kindly change "patent application 07/496,572" to --Example 1--.

At page 16, line 1 after "Example" change "3" to --6--.

At page 18, line 1 after "Example" change "4" to --7--.

At page 20, line 1 after "Example" change "5" to --8--.